ELSEVIER

Aquat. Living Resour. 15 (2002) 361–370

Aquatic Living Resources

www.elsevier.com/locate/aquliv

Effects of formalin chemotherapeutic treatments on biofilter efficiency in a marine recirculating fish farming system

Nicolas Keck [a,b], Guillaume Blanc [a,*]

[a] *UMR INRA/ENVN 1035 Chimiothérapie Aquacole et Environnement, Ecole Nationale Vétérinaire de Nantes, Route de Gachet, Atlanpole–La Chantrerie BP 40706, 44087, Nantes cedex 03, France*

[b] *Laboratoire Départemental Vétérinaire de l'Hérault, 306 rue Croix de Las Cazes, BP 6070, 34030 Montpellier, France*

Received 10 September 2001; accepted 30 October 2002

Abstract

Formalin (a 33–38% aqueous formaldehyde solution) is currently used for bath treatments to control ectoparasitic infections of fish. Its effects on nitrification were evaluated in a semi-closed pilot scale saltwater recirculating turbot culture system. Tested treatments included 1 h static exposure to 20, 30, 40, 50, 60, 70, 90 mg l^{-1} formaldehyde without flushing, and long-term (i.e., 2, 4 and 6 h) exposures with constant 60 mg l^{-1} formaldehyde in a recirculating system. Formaldehyde has no apparent effect on the ammonia oxidative bacterial community. However, a significant effect on nitrite oxidation was observed in 1 h static exposures of concentrations higher than 40 mg l^{-1}, and in recirculating system exposures of 60 mg l^{-1} formaldehyde for more than 4 h. Repeated treatments may be hazardous for nitrifying bacteria, which could induce an increase in nitrite concentration. Nitrite concentrations should be monitored when treatments are repeated or when they last a long time in recirculating aquaculture systems.

Résumé

Effets de traitements chimio-thérapeutiques au formol sur l'efficacité de biofiltres dans un système en circuit fermé d'élevage de poissons. Le formol (solution aqueuse de formaldéhyde à 33–38 %) est couramment utilisé en aquaculture pour des traitements thérapeutiques par balnéation afin de lutter contre les infestations des poissons par des parasites cutanés ou branchiaux. Ses effets sur les réactions de nitrification dans un système d'élevage pilote de turbot en circuit fermé ont été caractérisés. Des traitements statiques de formaldéhyde non suivis d'une vidange partielle des circuits fermés ont été appliqués pendant une heure à des concentrations de 20, 30, 40, 50, 60, 70 et 90 mg l^{-1}. L'effet de traitements dynamiques maintenant une concentration constante de 60 mg l^{-1} de formaldéhyde dans le circuit fermé pendant 2, 4 et 6 heures a également été évalué. Le formaldéhyde ne semble pas affecter les populations bactériennes dégradant l'ammoniac. En revanche, des traitements statiques à des concentrations de plus de 40 mg l^{-1} de formaldéhyde et des traitements dynamiques à une concentration de 60 mg l^{-1} pendant plus de 4 heures ralentissent la dégradation des nitrites par le filtre biologique. L'effet de traitements répétés pourrait avoir des conséquences néfastes sur le fonctionnement des biofiltres. La concentration en nitrites dans l'eau des systèmes d'élevage en circuit fermé devrait être rigoureusement contrôlée lorsque des traitements au formol sont répétés, ou qu'ils sont appliqués pendant une longue période.

Keywords: Recirculating system; Antiparasitic; Chemotherapy; Formalin; Nitrification; Nitrite; Turbot farming

1. Introduction

Water re-use technologies can benefit fish breeding by reducing water costs, heat loss, effluent volume and by making the treatment of a concentrated effluent more efficient. Many options are available to treat water and increase re-use rates: aeration and oxygenation by dissolved oxygen addition, sedimentation and mechanical filtration for suspended solids removal, de-gassing for nitrogen and carbon dioxide stripping, pH control and biological filtration for ammonia and nitrite removals (Colt and Orwicz, 1991).

* Corresponding author.
E-mail address: blanc@vet-nantes.fr (G. Blanc).

PII: S0990-7440(02)01198-1

These technologies have recently been considered mature and consistent large scale commercial applications have now been developed such as turbot (*Scophthalmus maximus*) recirculating farming in Brittany, France (Blancheton, 2000; Quemeneur et al., 2001).

Ammonia, the prominent form of nitrogenous wastes produced by ammoniotelic teleosts, may be highly toxic to aquatic organisms (Russo and Thurston, 1991; Wood, 1993). Ammonia removal is usually accomplished in biological filters (biofilters) through bacterial oxidation in a two-step process. In the first step, ammonia-oxidizing bacteria oxidize ammonia to nitrite, also toxic to fish, which is converted to nitrate by nitrite-oxidizing bacteria in the second step. Nitrate is considered relatively harmless to fish and can be maintained at safe levels with regular water changes (Hargreaves, 1998). Ammonia and nitrite concentrations are therefore among the most limiting factors in many aquafarming systems, due to the necessity of simultaneous management of equilibrated fish and bacterial populations (Pennell and McLean, 1996).

Although the bacterial species diversity which develops in biofilters is not precisely known (Austin, 1988), due to the differential kinetics of ammonia and nitrite removals, distinct microbial communities are stated to be responsible for these oxidative activities (Wheaton et al., 1991; Kikuchi, 1998). Among others, chemolithoautotrophic and heterotrophic bacteria belonging to genera such as *Nitrosomonas*, *Nitrosospira*, *Nitrospira*, *Nitrosococcus*, *Nitrobacter* and *Pseudomonas* were identified in ammonia and nitrite-oxidizing bacterial communities (Head et al., 1993; Stephen et al., 1996; Bartosch et al., 1999; Ivanova et al., 2000; Léonard et al., 2000).

Disease organisms may be favoured in these confined farming systems and in the intentionally bacteria-rich medium maintained in recirculating units, through which water is recycled. Since biofilters need the culture of large and slow-growing bacterial populations, disinfection management is difficult. Furthermore, antibiotic therapy to control fish bacterial diseases was identified as a major risk to biofilters (Collins et al., 1976; Klaver and Matthews, 1994). It may kill or impair the nitrifying bacteria of biofilters and, thus, entail a rise of ammonia and nitrite levels, prone to be toxic to fish (Arillo et al., 1981; Person-Le Ruyet et al., 1995, 1997a, b, 1998). Several alternative treatment processes have been considered such as ultraviolet light and intermittent ozonation (Rosenthal and Black, 1993). At this time, the effects of these treatments on fish ectoparasites are not accurately documented. Therefore, antiparasitic drugs and especially formalin are favoured by fish health specialists.

In turbot farming, the protozoans belonging to the genus *Trichodina* Ehrenberg, 1838 are considered as the most detrimental ectoparasites. Trichodinids are typically ectozoic commensals (Lom and Dykova, 1992). However, in recirculating systems or when the fish are debilitated, the trichodinids behave like serious ectoparasites and heavy trichodiniasis may cause significant direct mortalities in juvenile turbot. Probably much more common is the induced growth inhibition: up to 26% weight loss over a 12 month period has been reported in young cultured turbot (Sanmartin Duràn et al., 1991).

A large range of formalin regimens are currently used against trichodinids in turbot farming. More generally, in marine recirculating farming systems, formalin bath treatments are frequently employed in preventive or curative first choice treatments to control several species of protozoan and monogenean ectoparasites, although it sometimes appears insufficient to control the infection (Madsen et al., 2000).

The exposure of fish to high formalin levels can cause severe gill damage which can impair osmoregulation and respiratory activity. Formalin toxicity to fish increases at high temperatures, at low oxygen levels and at low water hardness. It can also vary with water salinity, fish species, strain, size and conditions (Piper and Smith, 1973; Reardon and Harrell, 1990; Meinelt and Stueber, 1992). In recirculating systems, it can also vary with the system design and thus the formalin loss. Nevertheless, up to 300 ppm formalin (approximately 100 mg l^{-1} formaldehyde) exposure can be used for treatments of the fingerlings of the grouper *Epinephelus coioides* (Yusoff and Mustaffa, 1998).

The inhibitory effect upon the nitrification process of the short-term formalin treatment has already been tested with no report of any significant change after treatments (Collins et al., 1975; Blancheton and Mélard, 1990; Wienbeck and Koops, 1990). However, in intensive conditions, a large range of formalin regimens including multidose preventive treatments are currently in use depending on the mean parasite burden. Therefore, the potential adverse effects of formalin on nitrification should be investigated at both various dosages for a single administration and for repeated treatments over a significant period. As treatments are often followed by the flush of most of the treated water inducing a loss of water and energy, the re-use effect of the treated water has to be evaluated. Hence, the consequences of various dose/time expositions of the biofilter bacteria to formalin deserve to be investigated.

The aims of this study were to determine the effects of various dose/time expositions of the biofilters bacteria on formalin upon nitrification by analysing the evolutions of nitrite and ammonia concentration and removal, through field recycling systems similar to those used in turbot fish farming conditions.

2. Materials and methods

The experiment was assessed from May to August 1997 in France Turbot fish farm (Noirmoutier, France).

2.1. Semi-closed test system: implementation and functioning

Three pilot scale recirculating semi-closed saltwater systems (F1, F2 and F3) were developed from critical volumetric and surface area relationships derived from the successful commercial system. Each pilot had a total volume of 1000 l and contained two tanks with a water volume of 300 l, a fluidized bed with Biospheres® (Bioinjection environnement, Beaupreau, France) as a media (130 l), a settling area followed by gravel filter for solids removal, an oxygenation system providing pure oxygen directly in the tanks and a de-gasser column. Water coming out of the biofilter was collected in a basin and it fell by gravity through the gas stripper before being distributed to the tanks. The total volume of each tank was renewed every hour. The water from the fish tank flowed through the mechanical filter and then into a pump delivering 660 l h^{-1} to the biofilter. Influent water obtained from the commercial scale system was brought at a volume representing 10% of the total volume every hour. The nitrifying bacteria came from already acclimated media removed from the active biofilters on the commercial scale turbot grow-out system. Backwash was performed every month using bubble washing.

Before starting the experiments, the correct functioning of the water re-use systems was checked, especially the stability of the bacterial populations which was evaluated by the stabilization of ammonia and nitrite concentrations.

2.2. Fish and breeding conditions

The system contained turbots from a commercial fish farm with starting stocking densities of 10 kg m^{-2} and a size range of approximately 30–40 g. They were fed three times a day with commercial fish food (Aqualife 54/15, Biomar, Nersac, France) at a rate of about 4.5% bw per day. Temperature and oxygen measured by an OxyGuard (Birkerød, Denmark) system, and pH were recorded twice a day (8 and 16 h) throughout the study. Oxygen was maintained over 80% saturation level, temperature between 18 and 24 °C, pH between 7.2 and 7.6 and salinity between 30 and 33 g l^{-1}.

2.3. Experimental design

After stable nitrification was demonstrated, different formalin treatments were applied in the F1 and F3 systems, F2 being used as a control during the study. In aquaculture chemotherapy, formalin is the generic name of a solution of 33–38% by weight of formaldehyde gas in water with 10–15% by weight of methanol as a stabilizing agent to delay polymerization (Scott, 1993). Diluted solutions are designated by their content of formalin or by the approximately equivalent formaldehyde concentrations. A 33% formaldehyde commercial solution was used in this study, and treatment concentrations are given as mg l^{-1} formaldehyde.

Table 1
Formaldehyde treatments performed in the pilot recirculating systems F1, F2 and F3

Treatment modalities	Concentration (mg l^{-1}) F1	F2	F3
A	30[a]	0	20[a]
B	50[a]	0	40[a]
C	60[a]	0	70[a]
D	90[a]	0	
E		0	60*
F	60***	0	60**

a Conditions of exposure: [a]static treatment, 1 h.
* Conditions of exposure: Star(s): dynamic treatment, *2 h, ** 6 h, ***4 h.

At first, 1 h treatments were performed every week at concentrations of 20, 30, 40, 50, 60, 70 and 90 mg l^{-1} formaldehyde. Formalin was kept in the tank for 1 h and then released in the recirculating system.

In the second part of the study, the entire system was exposed to longer term dynamic treatments (2, 4 and 6 h) at a constant formaldehyde concentration of 60 mg l^{-1} without stopping the water recirculation. This was realized by adding a formalin solution constantly to the water in order to compensate for the loss of the drug due to the water renewal.

These treatments were performed weekly after the correct functioning of biofilters had been checked. They are summarized in Table 1.

2.4. Analytical methods and investigated parameters

During the acclimation and the experimental periods, water samples were collected at the inlet and the outlet of each biofilter twice daily, just prior to feeding at 8 and 16 h. They were processed during the hour following the sampling; total ammonia nitrogen (TAN) and nitrite nitrogen ($N–NO_2$) were analysed.

TAN was measured by Koroleff (1969) colorimetric indophenol method and $N–NO_2$ by the Griess reaction adapted for seawater by Bendschneider and Robinson (1952). These techniques were adapted for the forage water used in our study by Moreau (1993).

Un-ionized ammonia (UIA-N) concentrations were calculated using the equation of Johansson and Wedborg (1980) which gives the ratio of UIA-N to TAN as a function of pH, temperature ($T = t$ °C + 273.2) and salinity (g l^{-1}): NH_3 (%) = $100/(1 + 10^{(pKa–pH)})$ where $pK_a = –0.467 + 0.0011S + 2887.9/T$ (1) Results are expressed in mg l^{-1} UIA-N (1 mmol l^{-1} equivalent to 17 mg l^{-1}) or in the equivalent TAN mg l^{-1} and in mg l^{-1} $N–NO_2$.

For each biofilter, the efficacy of removal of ammonia and nitrite was calculated as follows (Seo et al., 2001):

Efficacy of $N–NO_2$ removal = $([N–NO_2]_{in}–[N–NO_2]_{out})$ × water flow rate/media volume (2) Results are expressed in g $N–NO_2$ or TAN m^{-3} media h^{-1}. Fish growth was evaluated by weighing regularly a sample of the fish population (about 30 specimens).

2.5. Data analysis

TAN and $N–NO_2$ concentrations were first figured as time-series data, then mean values and associated standard errors were calculated for each dose/time combination of formalin exposure and each tank. In each pilot, TAN and $N–NO_2$ removals were determined and used as proxy indicators of the ammonia and nitrite oxidation efficiencies of the respective bacterial communities. Due to the non-normality of the data, Friedman non-parametric tests were used to compare the TAN and $N–NO_2$ concentrations as well as the efficacy of biofilters in each system. The level of significance was set at 5%. From the statistical analysis of TAN and $N–NO_2$ removals, the lowest combination of dose/time exposure to formaldehyde at which a significant effect on the biofilter efficacy was observed, was determined.

2.6. Risk of fish exposure to toxic levels of ammonia and nitrite

In the literature, the consequences of the exposure of fish to ammonia do seem multiple and prone to several significant covariates: ammonia toxicity depends primarily on pH and also on temperature, oxygen concentrations and the presence of other toxic substances (Russo and Thurston, 1991). Also, the LC50 increases with fish weight and acclimation to moderate concentrations of ammonia (Tudor et al., 1994). Reliable data for ammonia toxicity have been documented recently for defining marine environmental quality standards. In juveniles of turbot, the 96 h LC_{50} was estimated to be between 2.5 and 2.6 mg l^{-1} un-ionized ammonia (57–59 mg l^{-1} TAN) according to Person-Le Ruyet et al. (1995). Prolonged chronic exposure to ammonia causes major effects on reproductive capacity and disease resistance, on fish food intake and efficiency, on growth potential and body composition (Person-Le Ruyet et al., 1997a, 1998). The lowest observable effect concentration (LOEC) values for growth would be 0.41 mg l^{-1} un-ionized ammonia (15 mg l^{-1} TAN) for turbot weighing 14 g and 0.1 mg l^{-1} un-ionized ammonia (3.6 mg l^{-1} TAN) for 104 g turbot (Person-Le Ruyet et al., 1997b). Therefore, in this study, the risk of fish exposition to ammonia toxicity was estimated by the ratio between the ammonia level monitored in fish tanks to the lower range of the LOEC for fish in the size range of this study, i.e., 0.14 mg l^{-1} for a 53 g turbot (Person-Le Ruyet et al., 1997a).

Equally, the accumulation of nitrite in re-use systems can be toxic to aquatic organisms. The main fish health problem due to nitrite exposure is brown blood disease (methaemoglobinaemia) named after the brown-coloured methaemoglobin produced by nitrite oxidation of haeme group iron which cannot take in charge oxygen (Tomasso, 1986). Consequently, typical signs of methaemoglobinaemia are mostly similar to hypoxia and include melanosis, gill ventilation rates variations and lethargy. Other iron-containing enzymes can also be affected and Speare (1998) suggests that nitrite toxicity may also be immunosuppressive. Nitrite poisoning has been most extensively studied in channel catfish (*Ictalurus punctatus*) where firm recommendations regarding toxic levels can be made (Tucker et al., 1989). Data also exist for salmonids and cyprinids but for most species, they are lacking. The 96 h LC_{50} values found in the literature ranged from 0.19 to 1997 mg l^{-1} $N–NO_2$ (Scarano et al., 1984; Russo and Thurston, 1991; Parra and Yufera, 1999). Methaemoglobinaemia induction was demonstrated at concentrations 4–5 times lower than 96 h LC_{50} levels (Scarano et al., 1984) and little is known about long-term chronic effects of low concentrations. Furthermore, different parameters like exogenous chloride and oxygen concentrations, aquatic species, growth phase, pH and exposure time affect the tolerance of an organism for nitrite (Lewis and Morris, 1986; Bartlett and Neumann, 1998). It follows therefrom that in marine fingerlings of major cultured species the safe level is not known precisely. In this study, the risk of fish exposition to nitrite toxicity was weighted up by the ratio between the nitrite concentration monitored in fish tanks to 0.5 mg l^{-1} $N–NO_2$, the secure level suggested in mariculture practice (Blancheton, 2000).

3. Results

Over the experimental period (90 d), stocked animals increased in weight by 100%. Growth decreased at the beginning of July which corresponds with the period of high ammonia and nitrite concentrations generated by the experiments.

3.1. Water re-use system functioning

The pilot system was started and time was given to allow bacterial community development in biological filters. In normal functioning conditions, the stabilization of TAN and N–NO2 needed approximately 5 weeks (Fig. 1). TAN concentration first rapidly increased to values close to 3 mg l^{-1}, and then dropped, presumably due to establishment of ammonia oxidizers. Nitrite concentration subsequently decreased, as would occur with nitrite oxidizer settlement. Problems due to ill-functioning of de-gasser columns required their replacement and use of influent water (with low ammonia concentrations) to fill the system which entailed a drop of TAN concentrations around the sixth of May.

After the bacterial community had developed, TAN levels measured at 16 h at the outlet of the biofilters varied between 0.6 and 1.2 mg l^{-1} (Fig. 1) and inlet TAN

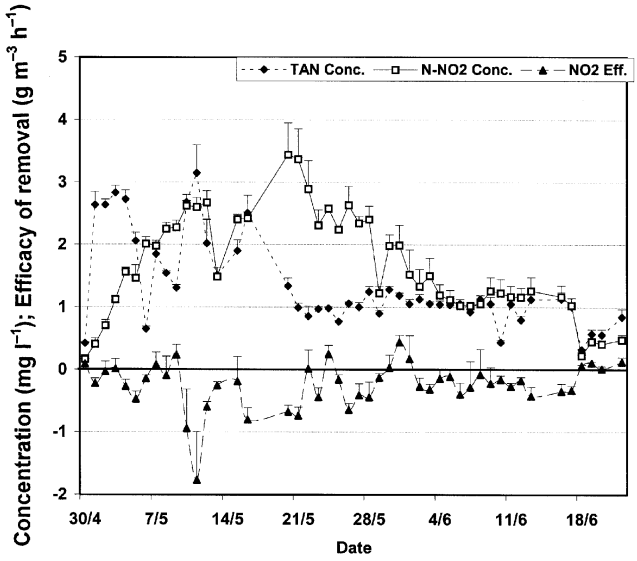

Fig. 1. TAN and N–NO_2 concentrations at the outlet of the biofilter and efficacy of nitrite removal during the development of bacterial community. Data used to draw this curve are the mean of the values monitored in the three studied recirculating systems for each parameter. Standard error lines are graphically represented on the graphic.

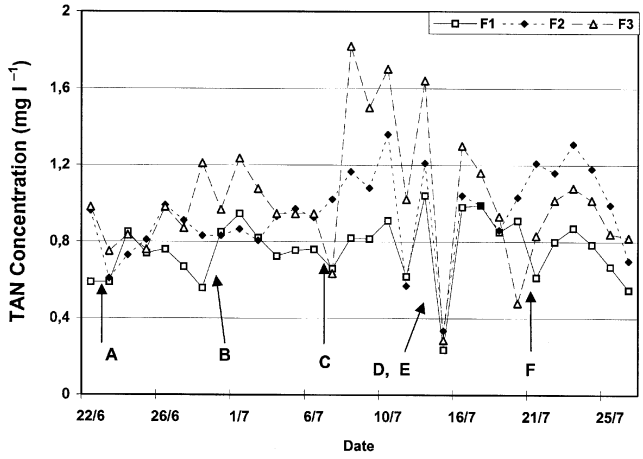

Fig. 2. Course of TAN concentrations at the outlet of each biofilter (F 1, 2 and 3) during the static 1 h and dynamic treatments period. Each arrow corresponds to one treatment (see Table 1).

concentrations were 0.8–1.8 mg l^{-1}. N–NO_2 levels measured at 16 h at the outlet of the biofilters varied between 0.5 and 1 mg l^{-1} (Fig. 1) and inlet N–NO_2 concentrations were 0.5–1 mg l^{-1}. The TAN concentrations at 16 h were 50–200% higher than at 8 h (data not shown).

During stabilization, the efficacy of TAN removal values fluctuated from 0.5 to about 3 g TAN m^{-3} media h^{-1} (data not shown). Values calculated for the efficacy of nitrite removal were initially very low (about −1 g N–NO_2 m^{-3} media h^{-1}) meanwhile those of N–NO_2 peaked. After that, they were more regular and remained almost zero, with a maximum around 0.4 g N–NO_2 m^{-3} media h^{-1} and a minimum at −0.4 g N–NO_2 m^{-3} media h^{-1} (Fig. 1). Negative nitrite removal values indicate that more nitrite was produced in the biofilter than was oxidized.

Factors such as water flow rate and dissolved oxygen level (Kaiser and Wheaton, 1983), pH (Alleman, 1985) and salinity range (Nijhof and Bovendeur, 1990) can affect nitrification. However, in the present study, these factors stayed within acceptable ranges for ammonia or nitrite oxidation.

3.2. *Effect of 1 h static treatment*

The effects of a 1 h formalin treatment at different concentrations on outlet ammonia concentration are shown in Fig. 2. Treatments regularly performed in the recirculating systems are represented on the graphic by an arrow and a letter corresponding to the formaldehyde concentration figured in Table 1. TAN concentration was generally lower after the treatment because fish received less food during that day but it did not increase significantly in the treated systems in comparison to the control. TAN levels stayed very low, between 0.6 and 1.8 mg l^{-1}, which are values monitored during the normal functioning period. The efficacy of removal decreased moderately (i.e., from 1 to 0.5 g TAN m^{-3} media h^{-1}) after treatments at concentrations beyond 40 mg l^{-1} (data not shown) but the effect subsided within a single day.

The distinctive course of N–NO_2 concentration and efficacy of removal in each recirculating system are shown in Fig. 3. The first administrations performed at concentrations below 40 mg l^{-1} (A) were not followed by any modification compared to the control. Adversely, the N–NO_2 concentration increased after a 40 mg l^{-1} treatment (B in F3) with a decrease of removal efficacy. After this, the N–NO_2 peaks were more and more intense when formaldehyde levels increased. This effect showed intense peaks in their levels (2.8 mg l^{-1} for treatment C, 70 mg l^{-1} in F3) and N–NO_2 levels reached highest values approximately 4 d after treatment and returned to baseline values 1 week after treatment. Nitrite concentrations after stabilization were close to 0.5 mg l^{-1}, i.e., values monitored during the routine functioning period, after the first acclimatization period of 5 weeks.

According to Friedman's test achieved with values concerning the static treatments period, the difference between the three systems during all the experiment is significant for N–NO_2 concentration ($P < 0.001$). The average range for concentration is much higher in F1 and F3 than in the control (Table 2); mean values of N–NO_2 concentration are superior in treated systems, 1.1 ± 0.5 mg l^{-1} (mean ± S.E.) for F1 after 60 mg l^{-1} treatment, 1.4 ± 0.8 mg l^{-1} for F3 after 70 mg l^{-1} treatment in comparison to the control, 0.9 ± 0.3 mg l^{-1}.

Therefore, the efficacy of nitrite removal between the three recirculating systems is much lower in treated systems than in control ($P < 0.001$). Lowest values (around −1.8 g N–NO_2 m^{-3} media h^{-1}) for F3 after treatment C were monitored the same day as the N–NO_2 peaks; the efficacy of

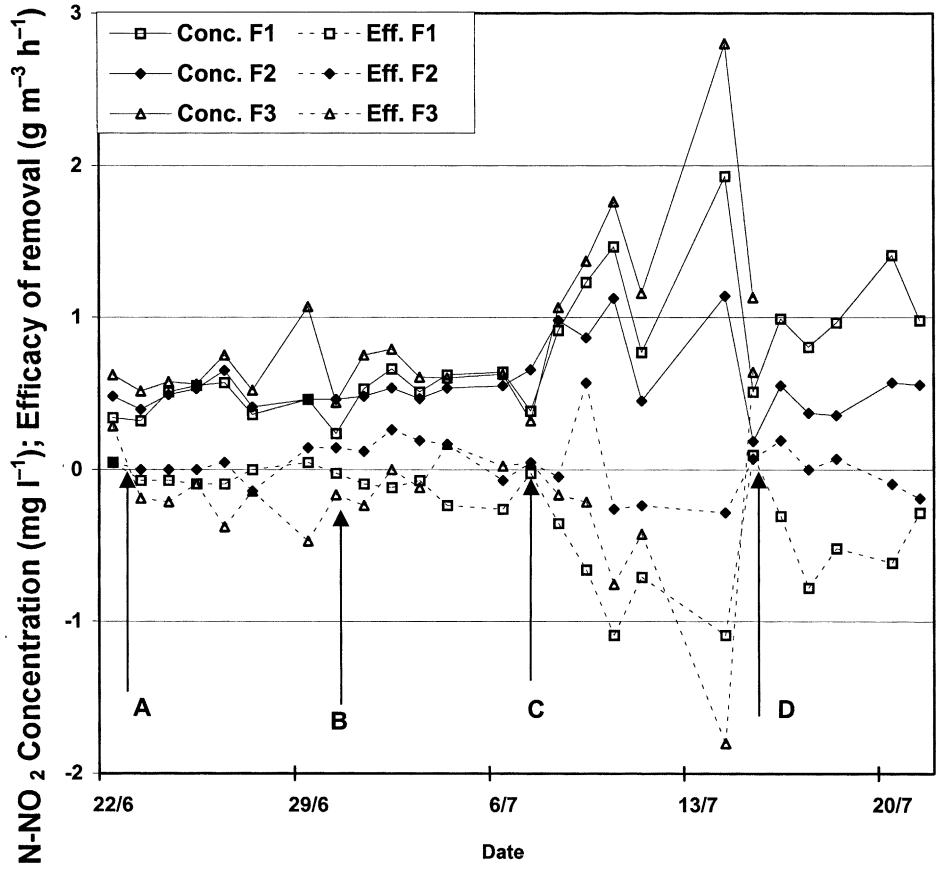

Fig. 3. Course of N–NO_2 concentrations at the outlet of each biofilter (F 1, 2 and 3) and evolution of the efficacy of removal for nitrite during the static 1 h treatment period. Arrows are labelling the treatment's modalities (see Table 1).

nitrite removal has approximately an inverse pattern of N–NO_2 concentration.

3.3. Dynamic treatments

The effects of a formalin dynamic treatment at different concentrations on outlet ammonia concentrations are illustrated in Fig. 2. TAN concentrations in treated systems after dynamic treatments were the same as in the control tank (F2) during the whole experiment. We did not even observe the drop of the efficacy of removal in treated systems that we made obvious during the previous experiment (data not shown).

The distinctive course of N–NO_2 concentration and efficacy of removal after dynamic treatments in each recirculating system are displayed in Fig. 4. N–NO_2 concentrations remained stable after a 60 mg l^{-1} treatment lasting 2 h (E in F3) but increased after 4 h (F in F3) and 6 h (F in F1) treatments and reached high values (between 1.5 and 2 mg l^{-1}) the third day after treatment. During this period, the efficacy of removal decreased in treated systems to approximately −1.2 g N–NO_2 m^{-3} media h^{-1} (treatments F) about 3 d after treatment, also exhibiting an inverse pattern compared to N–NO_2 concentrations. However, even if the effect of dynamic treatments shows the same pattern as static treatments (highest levels are reached approximately 3 d after exposition), the peaks were not as intense as those monitored during the first experiment.

According to Friedman's test achieved with values concerning the dynamic treatment period (Table 2), the difference between the three systems is significant during all the experiments for N–NO_2 concentrations ($P < 0.001$) and for

Table 2
Friedman's statistical test results comparing for each recirculating system data concerning N–NO_2 concentration and efficacy of removal after different formaldehyde concentrations (static and dynamic treatments periods)

Treatment type	N–NO_2	Average rank for Friedman's statistic			*P*-value	*n*
		F1	F2 (control)	F3		
One hour static	Concentration	45.5	32.5	66.3	<0.001	24
	Efficacy of removal	36.5	63.5	44	<0.001	24
Dynamic	Concentration	24	12	30	<0.001	11
	Efficacy of removal	15	27	24	<0.05	11

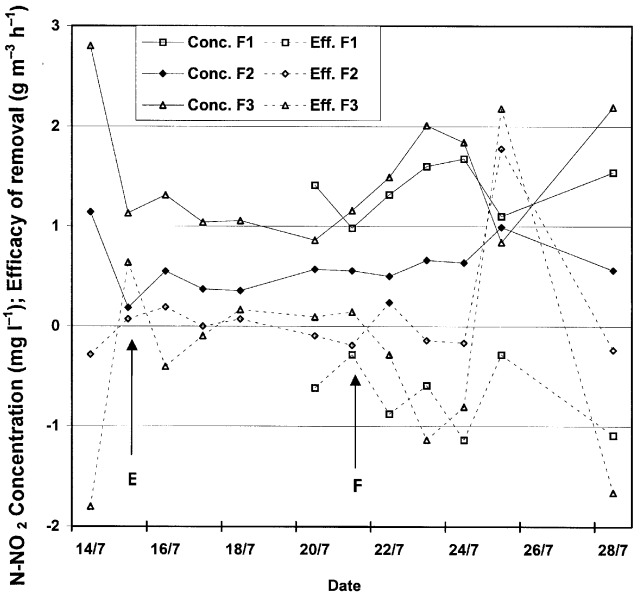

Fig. 4. Course N–NO_2 concentrations at the outlet of each biofilter (F 1, 2 and 3) and evolution of the efficacy of removal for nitrite during the dynamic treatments period. Arrows are labelling treatment's modalities (see Table 1).

efficacy of removal ($P < 0.050$). Hence, mean values of N–NO_2 concentrations in the treated systems are significantly higher when compared to the control. Mean N–NO_2 concentrations are 1.4 ± 0.3 mg l^{-1} (mean ± S.E.) for F1 after 60 mg l^{-1}, 6 h treatment and 1.6 ± 0.5 mg l^{-1} for F3 after 60 mg l^{-1}; 4 h treatment which are higher than the control (0.7 ± 0.2 mg l^{-1}).

During the treatments period, correlation statistical test analysing values monitored in all the recirculating systems showed that there was no significant relationship between the nitrite and the ammonia removal rates (coefficient of determination < 0.5, P value > 0.05), suggesting that these parameters were independent in our experimental conditions.

4. Discussion

The stabilization of TAN and N–NO_2 concentrations in our recirculating systems lasted a little more than a month; this delay agrees with observations made by other authors (Collins et al., 1976; Krüner and Rosenthal, 1983). Other pilot biofilter systems needed more time to reach steady state conditions for nitrifying populations (Greiner and Timmons, 1998). TAN concentration values checked at 16 h were 50–200% higher than values obtained in the morning which agrees with the observations made by Poxton and Allouse (1987). These authors observed a daily ammonia cycle showing peaks 73–205% greater than the pre-feeding concentration 3.5–4 h after feeding but also a weekly cycle reaching maximum concentrations at the end of Wednesday. In normal biofilters functioning conditions, the amount of oxidated nitrite is equivalent to the amount produced by ammonia oxidative bacteria. In our pilot biofilter systems, values measured concerning the nitrite efficacy of removal are close to zero at steady state (Fig. 1) which indicates that filters were functioning properly.

Formalin does not have any effect on ammonia oxidation. TAN concentration in the treated systems did not reach any peak after treatments and the decrease of ammonia removal observed after static treatments very soon subsided, as also reported by Blancheton and Mélard (1990), who observed a transient shift in ammonia level subsiding 4 d after three consecutive 350 ppm formalin daily treatments. Collins et al. (1975) did not observe any significant change after three indefinite 25 ppm applications of formalin, provided on alternate days. Wienbeck and Koops (1990) did not observe any significant effect for a 60 mg l^{-1} formaldehyde indefinite treatment either (not followed by flushing).

In contrast, nitrite concentrations increased after 1 h treatments not followed by flushing with ≥40 mg l^{-1} formaldehyde and after 4 h exposure or more to 60 mg l^{-1} formaldehyde in our recirculating system. Also, the height of N–NO_2 peaks increased with higher formalin levels, probably due to a greater effect of higher formalin concentrations. Furthermore, nitrites tended to accumulate in recirculating systems during approximately 3–4 d before their removal by the biological filters. Dynamic treatments had a more discrete effect suggesting that formalin degradation was faster in the recirculating system.

This evolution of nitrite concentrations has been observed neither by Collins et al. (1975) nor by Blancheton and Mélard (1990). However, these authors used lower formalin concentrations than those used in the present work or flushed the treated water after formalin administration. Although a similar increase of nitrite concentration after repeated treatments has been observed by Heinen et al. (1995) after three formalin indefinite treatments (i.e., without flushing) at 100, 100 and 70 ppm, these authors concluded upon a lack of practical concern for the operating system since formalin treatments are usually less frequent. Nevertheless, in our study, treatments were performed weekly to mimic the antiparasitic regimens implemented to control infestation by protozoan ectoparasites (Madsen et al., 2000).

In these conditions, the observed nitrite peaks, between 2 and 3 mg l^{-1}, have been attributed to a concentration-dependent direct impairment effect of formalin on nitrite-oxidizing bacteria. They may also result from a slow adaptation of the nitrite oxidizer community to higher substrate concentrations. However, ammonia concentrations checked twice a day were very low after treatments and, furthermore, we showed that the nitrites emergence was not correlated with a TAN removal rate increase. Also, nitrite-oxidizing bacteria are slow-growing bacteria with doubling times ranging from 12 to 32 h (Ehrich et al., 1995). Therefore, the setting up of bacterial biological filters takes several weeks and even moderate stress can lead to long-term modifications of the filters ecosystems. Hence, re-

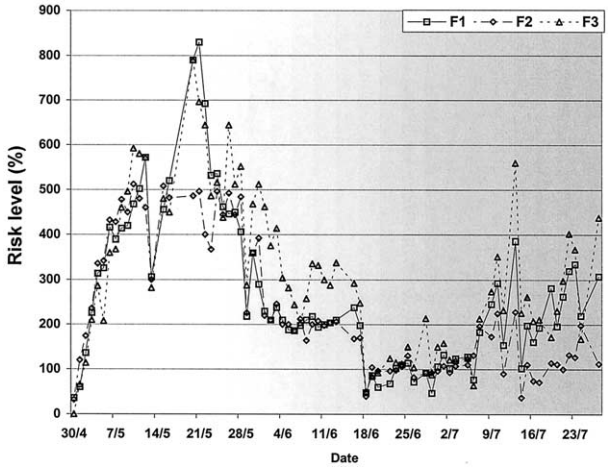

Fig. 5. Risk of fish exposition to nitrite toxicity during the study in the three recirculating systems. This ratio was calculated for the nitrite oxidizer settlement period (clear area) and the treatments period (dark area).

peated treatments as those conducted in this study, may modify the bacterial community in the biological filters, so that ammonia but not nitrite is eliminated.

The risk of fish exposition to ammonia and nitrite toxicity was evaluated during our study. Turbots were moderately exposed to ammonia toxicity during bacterial populations development. Afterwards, concentrations in our pilot systems never reached the toxic threshold for fishes. This low level of risk was favoured by the use of forage water with a very low pH (7–7.5) compared to marine water. On the contrary, fish were adversely exposed to nitrite toxicity during the settlement of bacterial communities (Fig. 5) and also after formalin treatments in relation with their negative effects on nitrification (Figs. 3–5). Although we did not observe any significant mortalities during the entire study, we noticed, however, a decrease of the fish growth rate associated with a lethargic behaviour of the treated fish when the levels of estimated risk were above 300% (Fig. 5), implying nitrite hazards to fish breeding and health (Tomasso, 1986; Speare, 1998).

In view of these results, nitrification and especially nitrite concentration should be monitored during repeated long-term formalin treatments periods. However, formaldehyde decomposition may fluctuate with water qualities because of its strong chemical reactivity, especially with compounds possessing a mobile hydrogen like proteins, nucleic acids or amino-acids (Kitamoto and Maeda, 1980). Therefore, the formaldehyde residence time in recirculating systems and the specific formaldehyde antiparasitic regimens capable of adverse effects on the nitrite-oxidizing bacterial community can likely vary with system design and management. Nevertheless, 1-h treatments not followed by flushing with ≥ 40 mg l^{-1} formaldehyde and a 4 h exposure or more to 60 mg l^{-1} formaldehyde should be considered as threshold values, triggering nitrite control procedures in marine recirculating systems.

5. Conclusion

From the data displayed in this paper, we suggest that the effect of formalin on nitrification is weak. It can entail a moderate increase of nitrite concentration reaching highest values approximately 4 d after treatments, exposing fish to a potential toxic effect. Fish farmers should monitor nitrite concentrations during and after repeated formalin treatments periods. This study could be completed by using simpler pilot scale recirculating systems which could make easier the control of every parameter influencing nitrification. Various data could be obtained using different pilot systems and fish species in order to feature mathematical modelling of the effect of chemotherapeutics on nitrification. Other chemotherapeutic agents, especially antibiotics including erythromycin (Collins et al., 1976), oxytetracycline (Klaver and Matthews, 1994), ampicillin, enrofloxacin, polymyxin B (Nimenya et al., 1999) have been shown to inhibit nitrification. Further studies concerning the possible effects of antibiotics on biofilters efficiency could be undertaken for extended periods (Skjolstrup et al., 2000) and repeated administrations. They could also provide an opportunity to study the antibioresistance levels within biofilters.

Acknowledgements

Authors have the pleasure to acknowledge helpful assistance from Mr Gildas Gauthier, Mr Didier Leclercq, France Turbot, Noirmoutier and an anonymous referee for critically reading the manuscript.

References

Alleman, J.E., 1985. Elevated nitrite occurrence in biological wasstewater treatment systems. Water Sci. Technol. 17, 409–419.

Arillo, A., Margoccio, C., Melodia, F., 1981. Ammonia toxicity mechanism in fish: studies on rainbow trout. Ecotoxicol. Environ. Safety 5, 316–328.

Austin, B., 1988. Marine Microbiology. Cambridge University Press, Cambridge.

Bartlett, F., Neumann, D., 1998. Sensitivity of brown trout alevins (Salmo trutta L.) to nitrite at different chloride concentrations. Bull. Environ. Contam. Toxicol. 60, 340–346.

Bartosch, S., Wolgast, I., Spieck, E., Bock, E., 1999. Identification of nitrite-oxidizing bacteria with monoclonal antibodies recognizing the nitrite oxidoreductase. Appl. Environ. Microbiol. 65, 4126–4133.

Bendschneider, K., Robinson, R.J., 1952. A new spectrophotometric method for the determination of nitrite in sea water. J. Mar. Res. 11, 87–96.

Blancheton, J.P., 2000. Developments in recirculation systems for Mediterranean fish species. Aquac. Eng. 22, 17–31.

J.P. Blancheton, J. Mélard, 1990. Effet des doses résiduelles de produits thérapeutiques les plus couramment utilisés en aquaculture marine sur le fonctionnement des filtres biologiques. GIE Recherche aquacole, MEREA, Fiche technique, Ref. JPB/DL 90.09.779.

Collins, M.T., Gratzek, J.B., Dawe, D.L., Nemetz, T.G., 1975. Effects of parasiticides on nitrification. J. Fish. Res. Board Can. 32, 2033–2037.

Collins, M.T., Gratzek, J.B., Shotts, F.B., Dawe, D.L., Mc Cambel, L., Senn, A.R., 1976. Effects of antibacterial agents on nitrification in an aquatic recirculating system. J. Fish. Res. Board Can. 33, 215–218.

Colt, J., Orwicz, K., 1991. Modeling production capacity of aquaculture systems under freshwater conditions. Aquac. Eng. 10, 1–29.

Ehrich, S., Behrens, D., Lebedeva, E., Ludwig, W., Bock, E., 1995. A new obligately chemolithoautotrophic nitrite-oxidizing bacterium Nitrospira moscoviensis sp. nov. its phylognentic relationship. Arch. Microbiol. 164, 16–23.

Greiner, A.D., Timmons, M.B., 1998. Evaluation of the nitrification rates of microbead tricking filters in an intensive recirculating tilapia production facility. Aquac. Eng. 18, 189–200.

Hargreaves, J.A., 1998. Nitrogen biochemistry of aquaculture ponds. Aquaculture 166, 181–212.

Head, I.M., Hiorns, W.D., Embley, T.M., McCarthy, A.J., Saunders, J.R., 1993. The phylogeny of autotrophic ammonia-oxidizing bacteria as determined by analysis of 16S ribosomal RNA gene sequences. J. Gen. Microbiol. 139, 1147–1153.

Heinen, J.M., Weber, A.L., Noble, A.C., Morton, J.D., 1995. Tolerance to formalin by a fluidized-bed biofilter rainbow trout Oncorhynchus mykiss in a recirculating culture system. J. World Aquac. Soc. 26, 65–71.

Ivanova, I.A., Stephen, J.R., Chang, Y.J., Bruggemann, J., Long, P.E., McKinley, J.P., Kowalchuk, G.A., White, D.C., Macnaughton, S.J., Chang, Y.J., 2000. A survey of 16S rRNA amoA genes related to autotrophic ammonia-oxidizing bacteria of the beta-subdivision of the class proteobacteria in contaminated groundwater. Can. J. Microbiol. 46, 1012–1020.

Johansson, O., Wedborg, W., 1980. The ammonia–ammonium equilibrium in seawater at temperature between 5 25 °C. J. Solut. Chem. 91, 37–44.

Kaiser, G.E., Wheaton, F.W., 1983. Nitrification filters for aquatic culture systems: State of the art. J. World Maricult. Soc. 14, 302–324.

Kikuchi, K., 1998. Water treatment techniques for closed recirculating aquaculture. Nippon Suisan Gakkaishi 64(2), 227–234.

Kitamoto, Y., Maeda, H., 1980. Reevaluation of the reaction of formaldehyde at low concentration with amino acids. J. Biochem. 87, 1519–1530.

Klaver, A.L., Matthews, R.A., 1994. Effects of oxytetracycline on nitrification in a model aquatic system. Aquaculture 123, 237–247.

Koroleff, C., 1969. Direct determination of ammonia in natural waters as indophenol blue. Int. Counc. Explor. Sea 9, 19–22.

Krüner, G., Rosenthal, H., 1983. Efficiency of nitrification in trickling filters using different substrates. Aquac. Eng. 2, 49–67.

Léonard, N., Blancheton, J.P., Guiraud, J.P., 2000. Populations of heterotrophic bacteria in an experimental recirculating aquaculture system. Aquac. Eng. 22, 109–120.

Lewis, M.L., Morris, D.P., 1986. Toxicity of nitrite to fish: A Review. Trans. Am. Fish. Soc. 115, 183–195.

Lom, J., Dykova, I., 1992. Protozoan Parasites of Fishes. Developments in Aquaculture, Fisheries Science. Elsevier Science, Amsterdam 26.

Madsen, H.C.K., Buchmann, K., Mellergaard, S., 2000. Treatment of trichodiniasis in eel (Anguilla anguilla) reared in recirculation systems in Denmark: alternatives to formaldehyde. Aquaculture 186, 221–231.

Meinelt, T., Stueber, A., 1992. Subchronic fish toxicity of formalin baths on eggs fry in dependence of water hardness. Fisch. Teichwirt 43, 294–295.

C. Moreau, 1993. Caractéristiques et gestion des eaux souterraines salées dans la baie de Bourgneuf: synthèse des connaissances et standardisation de protocoles communs d'analyse des paramètres de qualité. Syndicat mixte pour le développement aquacole en Pays de Loire. Laboratoire de Biologie marine, Université de Nantes.

Nijhof, M., Bovendeur, J., 1990. Fixed film nitrification characteristics in sea-water recirculation fish culture systems. Aquaculture 87, 133–143.

Nimenya, H., Delaunois, A., La Duong, D., Bloden, S., Defour, J., Nicks, B., Ansay, M., 1999. Short-term toxicity of various pharmacological agents on the in vitro nitrification process in a simple closed aquatic system. Alternatives Lab. Animals 27, 121–135.

Parra, G., Yufera, M., 1999. Tolerance response to ammonia nitrite exposure in larvae of two marine fish species (gilthead seabream Sparus aurata L. Senegal sole Solea senegalensis Kaup). Aquac. Res. 11–12, 857–863.

Pennell, W., McLean, W.E., 1996. Early rearing. In: Pennell, W., Barton, B.A. (Eds.), Principle of Salmonid Culture, Developments in Aquaculture and Fisheries Science. Elsevier Science, Amsterdam, pp. 365–451.

Person-Le Ruyet, J., Bœuf, G., Zambonino Infante, J., Helgason, S., Le Roux, A., 1998. Short-term physiological changes in turbot and seabream juveniles exposed to exogenous ammonia. Comp. Biochem. Physiol. 119A (2), 511–518.

Person-Le Ruyet, J., Delbard, C., Chartois, H., Le Delliou, H., 1997a. Toxicity of ammonia to turbot juveniles: 1. Effects on survival growth food utilisation. Aquat. Living Resour. 10, 307–314.

Person-Le Ruyet, J., Galland, R., Le Roux, A., Chartois, H., 1997b. Chronic ammonia toxicity in juvenile turbot (Scophthalmus maximus). Aquaculture 154, 155–171.

Person-Le Ruyet, J., Chartois, H., Quemener, L., 1995. Comparative acute ammonia toxicity in marine fish plasma ammonia response. Aquaculture 136, 181–194.

Piper, R.G., Smith, C.E., 1973. Factors influencing formalin toxicity in trout. Progress. Fish Cult. 35, 78–81.

Poxton, M.G., Allouse, S.B., 1987. Cyclical fluctuation in ammonia nitrite nitrogen resulting from the feeding of turbot Scophthalmus maximus in recirculating system. Aquac. Eng. 6, 301–322.

Quemeneur, F., Jaquen, P., Maleriat, J.P., Schlumpf, J.P., Bon, L., Lebegue, E., 2001. Techniques à membranes appliquées à l'élimination des matières en suspension dans un circuit semi-fermé d'aquaculture. Rev. Sci. Eau 1, 21–34.

Reardon, I.S., Harrell, R.M., 1990. Acute toxicity of formalin copper sulfate to striped bass fingerlings held in varying salinities. Aquaculture 87, 255–270.

Rosenthal, H., Black, E.A., 1993. Recirculation systems in aquaculture. In: Wang, J.K. (Ed.), Techniques for Modern Aquaculture. Am. Soc. Agricult. Eng. St. Joseph, MI, pp. 284–294.

Russo, R.C., Thurston, R.V., 1991. Toxicity of ammonia, nitrite and nitrates to fishes. In: Brune, E., Tomasso, J.R. (Eds.), Aquaculture and Water Quality. World Aquacult. Soc. Publ., Baton Rouge, LA, pp. 58–89 vol. 3.

Sanmartin Duràn, M.L., Fernandez Casal, J., Tojo, J.L., Santamarina, M.T., Estevez, J., Ubeira, F., 1991. Trichodina sp.: effects on the growth of farmed turbot (Scophthalmus maximus). Bull. Eur. Assoc. Fish Pathol. 11, 89–91.

Scarano, G., Saroglia, M.G., Gray, R.H., Tibaldi, E., 1984. Hematological response of seabass Dicentrarchus labrax to sublethal nitrite exposure. Trans. Am. Fish. Soc. 113, 360–364.

Scott, P., 1993. Therapy in aquaculture. In: Brown, L. (Ed.), Aquaculture for Veterinarians: Fish Husbandry, Medicine. Pergamon Press, Oxford, pp. 131–152.

Seo, J.K., Jung, I.H., Kim, M.R., Kim, B.J., Nam, S., Kim, S.K., 2001. Nitrification performance of nitrifiers immobilized in PVA (polyvinyl alcohol) for a marine recirculating aquarium system. Aquac. Eng. 24, 181–194.

Skjolstrup, J., McLean, E., Nielsen, P.H., Frier, J.O., 2000. The influence of dietary oxolinic acid on fluidised bed biofilter performance in a recirculation system for rainbow trout (Oncorhynchus mykiss). Aquaculture 183, 255–268.

Speare, D.J., 1998. Non-infectious disorders associated with intensive aquaculture husbandry. In: Leatherland, J.F., Woo, P.T.K. (Eds.), Fish Diseases and Disorders, vol. 2: Non-infectious Disorders. CAB Publishing, Wallingford, pp. 303–333.

Stephen, J.R., McCaig, A.E., Smith, Z., Prosser, J.I., Embley, T.M., 1996. Molecular diversity of soil and marine 16S rRNA gene sequences related to beta-subgroup ammonia-oxidizing bacteria. Appl. Environ. Microbiol. 62, 4147–4154.

Tomasso, J.R., 1986. Comparative toxicity of nitrite to freshwater fishes. Aquat. Toxicol. 8, 129–137.

Tucker, C.S., Francis-Floyd, R., Beleau, M.H., 1989. Nitrite-induced anemia in channel catfish Ictalurus punctatus Rafinesque. Bull. Environ. Contam. Toxicol. 43, 295–301.

Tudor, M., Katavic, I., Marsic-Lucic, J., 1994. Acute toxicity of ammonia to juvenile sea-bass (Dicentrarchus labrax L.) at different aeration levels. Aquaculture 128, 89–95.

Wheaton, F.W., Hochheimer, J., Kaiser, G.E., 1991. Fixed film nitrification filters for aquaculture. In: Brune, E., Tomasso, J.R. (Eds.), Aquaculture and Water Quality. World Aquacult. Soc. Pub., Baton Rouge, LA, pp. 272–303 vol. 3.

Wienbeck, H., Koops, H., 1990. Decomposition of formaldehyde in a recirculation fish farming system. Arch. Fisch. Wiss. 40, 153–166.

Wood, C.M., 1993. Ammonia urea metabolism excretion. In: Evans, D.H. (Ed.), The Physiology of Fishes. CRC Press, Boca Raton, FL, pp. 379–425.

Yusoff, N.H.N., Mustaffa, S., 1998. Acute toxicity pathological changes in gills of *Epinephelus coioides* juveniles to formalin. Food Res. Inst. Newsl. 4, 12.